Incorporation of [2-^{14}C]Mevalonic Acid into the Prototype Sterol 3β-Hydroxyprotosta-17(20),24-diene† with Cell-free Extracts of *Emericellopsis* Sp.

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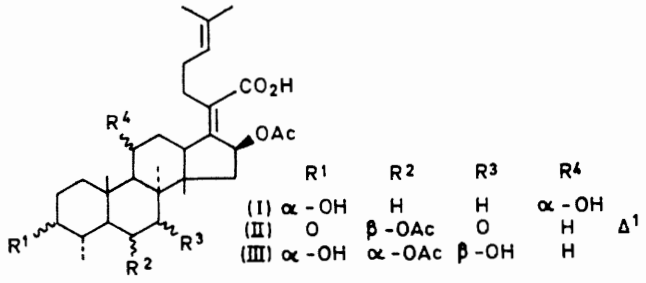
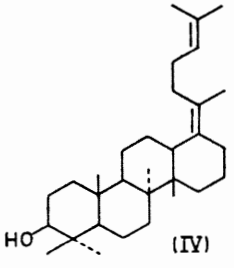
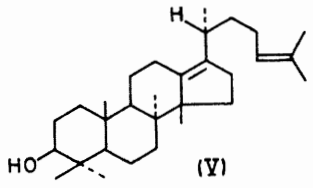
Summary Cell-free extracts of *Emericellopsis salmosynnemata* Grosklags et Swift converted [2-^{14}C]MVA into 3β-hydroxyprotosta-17(20),24-diene in 0·27% yield.

RECENT rapid progress in the studies on 2,3-oxidosqualene cyclase[1] and on the biogenesis of fusidic acid (I)[2,3] prompted





(IV)



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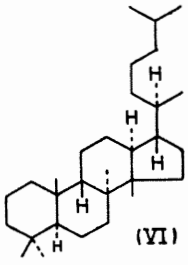

(VI)

us to undertake the preparation of a cell-free system for biosynthesizing 3β-hydroxyprotosta-17(20),24-diene (IV),[4] which is a principal intermediate in the biosynthetic processes of (I) and related antibiotics such as helvolic acid (II)[5] and cephalosporin P_1 (III).[6]

Eleven helvolic acid-producing micro-organisms were tested for this purpose (see Table). The cell-free extracts of

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Expt. No.	Enzyme source	Radioactivity in nonsaponifiable fraction (d.p.m.)	Conversion ratio (%)
1.	*Emericellopsis mirabilis* (Malan) Stolk NHL 2417	53,720	0·57
2.	*E. synnematicola* Mathur et Thirumalachar IFO 8519	132,960	1·42
3.	*E. humicola* (Cain) Gilman IFO 8518	93,320	0·99
4.	*E. synnematicola* Mathur et Thirumalachar CBS 176·60	28,880	0·31
5.	*E. salmosynnemata* Grosklags et Swift IFO 8517	126,640	1·35
6.	*E. microspora* Bachus et Orpurt NHL 2412	134,500	1·44
7.	*E. microspora* Backus et Orpurt NHL 2414	9240	0·10
8.	*Asperigillus fumigatus* Fres. C-95	1740	0·02
9.	*Cephalosporium caruleus*	26,640	0·28
10.	*Fusidium coccineum* Fuckel IFO 6813	6640	0·07
11.	*Fusarium oxysporum f. niveum* IFO 4471	1380	0·02

[a] Mycelia (10 g) were ground in a mortar with sea sand (10 g) and the mixture was treated with 0·1 M-potassium phosphate buffer, pH 7·4 (10 ml). The extract was centrifuged at 10,000 × *g* for 10 min. The supernatant was employed as the cell-free extracts.

[b] The reaction mixture contained the following components in a final volume of 1·0 ml: ATP, 2·0 mg; GSH, 1·0 mg; NADP, 0·3 mg; G6P, 3·0 mg; $MgSO_4$, $7H_2O$, 0·3 mg; $MnSO_4$, $4H_2O$, 0·2 mg; [2-^{14}C]MVA (9·4 × 10^6 d.p.m.); 0·5 ml of cell-free extracts. Reactions were carried out at 37° for 2 hr.

[c] The reaction mixture was saponified with 1 ml of CH_3OH and 0·3 g of KOH at 70° for 2 hr. under an atmosphere of N_2. The nonsaponifiable fraction (NSF) was extracted with ether.

three micro-organisms of *Emericellopsis* sp. converted more than 1% of added [2-^{14}C]MVA into nonsaponifiable fraction (NSF), which was found to consist mainly of two components—lanosterol fraction and squalene—by radioscanning of its thin-layer chromatogram on silica gel. In Expts. 2 and 6, squalene was the major product, and in Expt. 5, the lanosterol fraction predominated. The labelled sterols produced in Expt. 5 were characterized by the dilution method with lanosterol (VII), 3β-hydroxyprotosta-17(20),24-diene (IV),[4] and 3β-hydroxyprotosta-13(17),24-diene (V).[4] The sterol fraction from silica gel column chromatography of NSF was divided into three portions and to each portion 10 mg of the respective sterol was added. The mixtures were treated with benzoyl chloride–pyridine and the benzoates thus obtained were purified by repeated crystallization to obtain a specimen with constant specific radioactivity. Incorporation ratios of [2-^{14}C]MVA into (VII) and (IV) were 0·54 and 0·27%, respectively. However, no activity was observed in the case of (V). The total activities of (VII) plus (IV) were 80% of the total activities of the sterol fraction.

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† Since (IV) and (V) are considered to be prototype derivatives of sterols, we have used the name "protostane" in this communication for the skeleton (VI).[4]

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